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ORIGINAL PAPER

IMPACT OF VERMICOMPOST ON BIOLOGICAL INDICATORS OF THE QUALITY OF SOIL UNDER MAIZE IN A GREENHOUSE EXPERIMENT*

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Abstract

The objective of this study was to determine the effect of vermicompost application on microbial biomass carbon (MBC), dehydrogenase activity (DHA), and the functional diversity of microbial communities (BIOLOG assay using EcoPlates™) in the cultivation of maize. The greenhouse experiment included variants: control (no fertilizer), CAN (mineral fertilizer calcium ammonium nitrate 60 kg N ha^{-1}), V20+CAN (vermicompost 20 t ha^{-1}+mineral fertilizer 30 kg N ha^{-1}), V40 (vermicompost 40 t ha^{-1}) and V80 (vermicompost 80 t ha^{-1}). Soil samples were analyzed at the start and end of the experiment (after 74 days). More pronounced influence of fertilization on biological parameters was found at the end than at the start of the experiment. MBC was statistically significantly higher in variants V40 (14.8%) and V80 (32.4%) than in the control. The application of CAN had a significantly negative effect, but the combination of V20+CAN positively influenced dehydrogenase activity. However, soil BIOLOG data indicated that the Shannon diversity index and evenness were significantly the lowest in the V20+CAN variant. The study suggests that vermicompost improves soil quality, and represents a suitable alternative to mineral fertilizers in the cultivation of maize.

Keywords: fertilization, microbial biomass carbon, dehydrogenase activity, functional diversity, *Zea mays* L.

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INTRODUCTION

Conventional agriculture is currently characterized by excessive inputs of mineral fertilizers and pesticides, and insufficient application of organic fertilizers (Gill, Garg 2014, Guo et al. 2015). However, continuous and excessive application of mineral fertilizer was found to cause soil degradation (Ju et al. 2009) and environmental pollution (water, soil and food). Soil microorganisms are sensitive to changes in the management of farming systems and fertilization regimes can also affect the population, structure and function of soil microorganisms (Hu et al. 2011). Therefore, soil microbiological and biochemical properties such as microbial biomass, community proportion, metabolic activity, functional diversity, and various enzymatic activities are often measured to provide immediate and accurate information about small changes in soils (Bending et al. 2004, Bhattacharyya et al. 2005, Islam et al. 2011).

Vermicomposting is an alternative procedure through which we can get good quality organic fertilizer from organic residues (Roy et al. 2010). Vermicompost has a much finer structure than composts (Edwards 1998), and has a large particulate surface area that provides many microsites for microbial activity and strong retention of nutrients. Vermicompost is rich in highly diverse microbial populations, particularly fungi, bacteria and actinomycetes, in addition to which it contains plant growth regulators and other growth--influencing materials produced by microorganisms (Atiyeh et al. 2002). Some authors attribute the positive effect of vermicompost on plant growth and productivity of plants (Kale et al. 1992, Sinha et al. 2009) to vermicompost acting as a source of plant growth regulators like cytokinins, auxins, abscisic acid, gibberellins, phenolic acids (Aremu et al. 2015) produced by interactions between microorganisms and earthworms. Atiyeh et al. (2000) showed that vermicomposts have the potential for improving plant growth when added to greenhouse container media or soil, although the authors concluded that there are differences between vermicomposts in terms of their nutrient content, the nature of their microbial communities, and their effects on plant growth.

This study was completed to assess the fertilization effect of vermicompost on soil quality by monitoring changes in selected biological properties of soil.

MATERIAL AND METHODS

Soil and experimental design

The soil for the greenhouse experiment was obtained from a cadastral area of the municipality Banín (49°40′24.95″N; 16°27′26.13″E), the Czech Republic. This soil is considered to be vulnerable in terms of the potential

loss of soil N. The soil was classified as sandy-loam Fluvisol modal. The soil contained 13.0 g kg^{-1} of organic carbon, determined by the Tiurin wet oxidation method (Dziadowiec, Gonet, 1999), 1.410 g kg^{-1} of total nitrogen, estimated with the Kjeldahl method, 138 mg kg^{-1} P and 370 mg kg^{-1} K, determined according to Zbíral (2002), Mehlich (2008). pH/H_2O was 6.47 (determined potentiometrically in soil-water suspension in the 1 : 2.5 ratio) and pH/$CaCl_2$ 5.62 (in soil-0.1 mol l^{-1} $CaCl_2$ suspension in the 1 : 5 ratio).

Batches of 14.6 kg of soil were placed in individual experimental pots that were planted with *Zea mays* L. (ASTERI CS, FAO 240) and kept in a growth chamber at 26°C (day temp.), 20°C (night temp.) and 65% humidity, with a day length of 11 h (light intensity 3 000 lx). Irrigation doses were determined on the basis of the actual soil moisture, which was maintained at 70% of water holding capacity. Five variants of the experiment were prepared: Control (no fertilizer), CAN (addition of mineral fertilizer calcium ammonium nitrate at a dose 60 kg N ha^{-1}), V20+CAN (addition of vermicompost at a dose 20 t ha^{-1} and CAN at a dose 30 kg N ha^{-1}), V40 (addition of vermicompost at a dose 40 t ha^{-1}) and V80 (addition of vermicompost at a dose 80 t ha^{-1}). Vermicompost was produced from compost consisting of cattle manure (30%), sheep manure (25%), cereal straw (10%), biodegradable waste (grass, leaves, lop etc. 25%) and soil (10%) fermented for 12 months. After that period, compost was enriched with earthworms (*Eisenia foetida*) and fermented for 120 days.

The properties of vermicompost were as follows: the content of organic carbon 132.6 g kg^{-1}; the content of total nitrogen 14.86 g kg^{-1} and pH/H_2O 7.53. Analyses of these characteristics were made with the same methods as applied to soil samples. Mineral fertilizer Calcium ammonium nitrate (CAN) contains 27% of total nitrogen (nitrate and ammonium nitrogen ratio is 1:1), 4.1% of total MgO, about 7% of total CaO and 2% of CaO soluble in water (producer: Duslo, a.s. Šaľa, Slovak Republic).

Analysis

At the start (immediately after adding fertilizers) and end of the experiment (after 74 days), the following soil parameters were determined: microbial biomass carbon (MBC) by fumigation-extraction method (Vance et al. 1987) and dehydrogenase activity by the method of Casida et al. (1964).

The functional diversity of soil microbial communities was measured with a BIOLOG assay using EcoPlates™ (Biolog® Inc., Hayward, CA, USA). Microbial activity, expressed as the average well colour development (AWCD), was determined according to Garland (1996), from the formula: AWCD = $\sum OD_i/31$, where OD_i is optical density values from each well, corrected by subtracting the blank well values from each plate well (Garland, Mills 1991).

The soil microbial community structure and functional diversity were determined using the Shannon diversity index, substrate evenness and rich-

ness (Zak et al. 1994, Staddon et al. 1997). The Shannon index was derived form $H' = - \sum pi$ (ln pi), and pi was calculated by subtracting the control from each substrate absorbance and then dividing this value by the total colour change recorded for all 31 substrates. Substrate evenness was calculated as $E = H'/\ln$ (richness), where richness referred to the number of substrates utilized (Zak et al. 1994). According to their chemical nature, the substrates were divided into six substrate categories, i.e. carbohydrate, carboxylic acids, amino acids, miscellaneous, polymers and amines/amides (Preston-Mafham et al. 2002), and total absorbance of each category was calculated.

Statistical analysis

Statistical analysis of variance (ANOVA) was used to evaluate the impact of vermicompost on the measured parameters, and least significant differences (LSD) were used to compare means at the level of significance 0.05 ($P < 0.05$) (Statgraphics XV.). Principal component analysis (PCA), which is the most commonly used ordination technique for a BIOLOG data analysis, was calculated with the help of MVSP 3.13r (Multi-Variate Statistical Package 3.13r, Kovach Computing Services).

RESULTS AND DISCUSSION

Biological indicators of soil quality are parameters that are sensitive to changes in the soil. The soil microbial biomass carbon (MBC) is regarded as one of the most sensitive indicators of the sustainability of a management system (Gregorich et al. 1994).

At the start of the greenhouse experiment (immediately after adding fertilizers) we found the MBC (Figure 1A) within the range of 412.5 to 644.5 μg g^{-1}. MBC was significantly higher than in the control only after the application of vermicompost at a dose of 80 t ha^{-1}. However, this state can be considered temporary. Increasing the microbial biomass carbon is possible through the direct effect of the microorganisms added with vermicompost; also, the microorganisms added to the soil must be adapted to the environment. This could explain the increase in MBC only in the variant with the highest dose of vermicompost. As reported by Marinari et al. (2007), a significant increase of microbial biomass carbon in vermicompost-treated soils may also be due to an improved availability of substrate C that stimulates the microbial growth.

During the growing season of maize, the content of nutrients available to soil microorganisms increased by adding root exudates (sugars, organic acids, amino acids, flavonoidy, carbohydrates etc.) (Jacoby et al. 2017). This could be the cause of significant differences in the microbial biomass carbon content between analyses at the beginning and the end of the experiment. At the end of the greenhouse experiment, differences in the MBC content

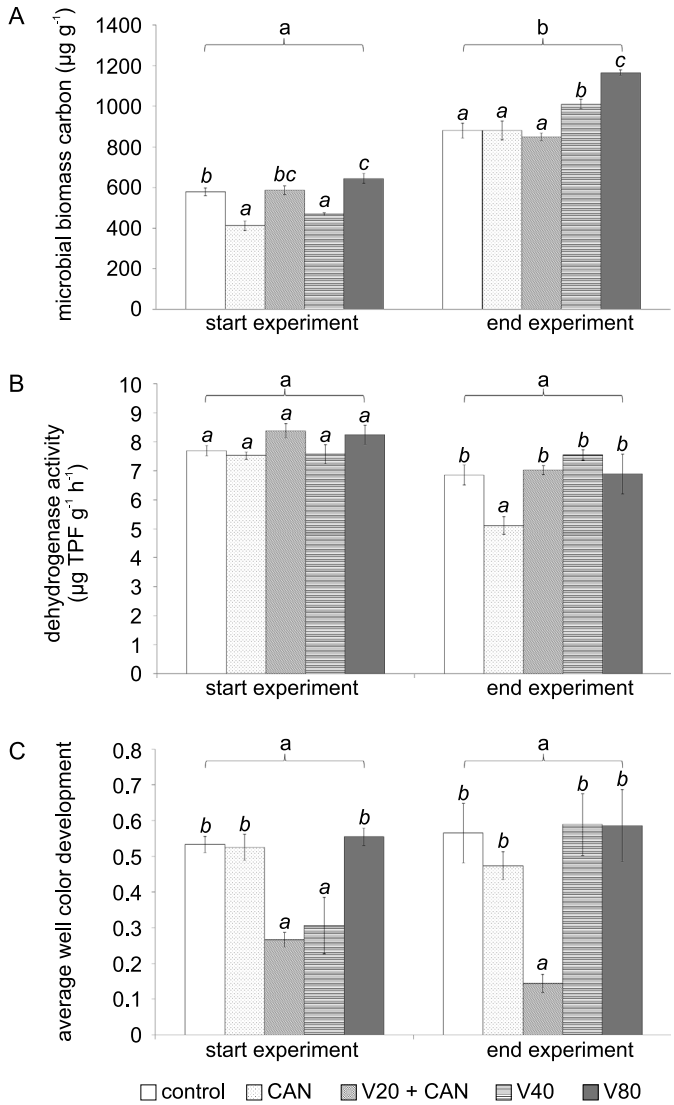

Fig. 1. Microbial biomass carbon (A), dehydrogenase activity (B) and average well colour development (C) start experiment – the letter above the column V80 is badly visible. Columns with different letters are significantly different at $P < 0.05$. The error bars indicate the standard error of the mean ($n = 3$); control – no fertilizer; CAN – mineral fertilizer 60 kg N ha^{-1}; V20+CAN – vermicompost 20 t ha^{-1} + mineral fertilizer 30 kg N ha^{-1}; V40 – vermicompost 40 t ha^{-1}; V80 – vermicompost 80 t ha^{-1}

between 849.1 and 1165.9 µg g^{-1} were observed, corresponding to the different vermicompost treatments (Figure 1A). The MBC was statistically significantly higher in the vermicompost treatment with the dose 80 t ha^{-1} (1165.9 µg g^{-1}) compared to the control (about 32.4% higher) and also to other

treatments. A significantly lower MBC content than in response to V80, but higher than in the control (about 14.8%), was found in the vermicompost treatment with the dose 40 t ha^{-1} (1010.8 µg g^{-1}). In agreement with our results, Arancon et al. (2005) also reported significantly higher ($P \leq 0.05$) microbial biomass carbon in soil treated with vermicompost (from cattle manure, food waste, and paper waste) than in soil with mineral fertilizer in pepper fields experiment. Our results confirm that the addition of vermicompost at a dose of 20 t ha^{-1} in a mix with mineral fertilizers was insufficient to increase microbial biomass carbon.

Among many biological properties that can serve as sensitive indicators of soil quality, enzyme activities often provide a unique integrative biological assessment of soil function, especially the ones catalysing a wide range of soil biological processes, such as dehydrogenase (Gil-Sotres et al. 2005, Mijangos et al. 2006).

Changes in dehydrogenase activity (DHA) that were due to the soil treatments are shown in Figure 1B. At the start of the experiment, dehydrogenase activity values were from 7.53 to 8.24 µg TPF g^{-1} h^{-1}. Our results suggested that vermicompost does not increase DHA immediately after application. Arancon et al. (2006) confirmed significantly lower DHA occurring in vermicompost-treated plots at the start of an experiment with strawberry plants. It may also have been due to an inhibitory effect resulting from the introduction of ´foreign´ soil microorganisms from the vermicomposts to the exotic microflora, which might have triggered competition among microorganisms. At the end of the experiment, DHA values after fertilization with vermicompost (all variants) were higher than in the untreated control, but the differences between were not statistically significant. Dehydrogenase activity was not significantly different from the control, presumably due to the humified organic matter added with vermicompost, which is more resistant to microbial mineralization. In a soil amendment with municipal solid waste compost, the same results were reported by García-Gil et al. (2000). We confirmed the negative effect of mineral fertilizer (CAN, dose 60 kg N ha^{-1}) on DHA. At the end of the experiment, the values of DHA were about 25.4% lower than in the control. Masciandaro et al. (2000) reported that mineral fertilizer reduced the enzyme-substrate affinity or changed the composition and activity of soil microbiota while vermicompost did not alter this affinity. In contrast, in the long-term (59 years) application of mineral NPK, Šimon and Czakó (2014) observed no inhibitory effect to DHA. We confirmed the positive effect of mixing CAN and vermicompost on DHA. Similarly, Srivastava et al. (2012) reported an increase in DHA upon vermicompost application to soil (dose 10 t ha^{-1}) and in the mixing of vermicompost+NPK (vermicompost 5 t ha^{-1} + NPK dose 50:40:40) with significant difference ($P < 0.05$) in comparison to mineral fertilizer (NPK, dose 100:80:80). In their experiment, DHA was found to be 71% higher in the mixing treatment as compared to the mineral fertilizer NPK. In our experiment, it was 27.21%.

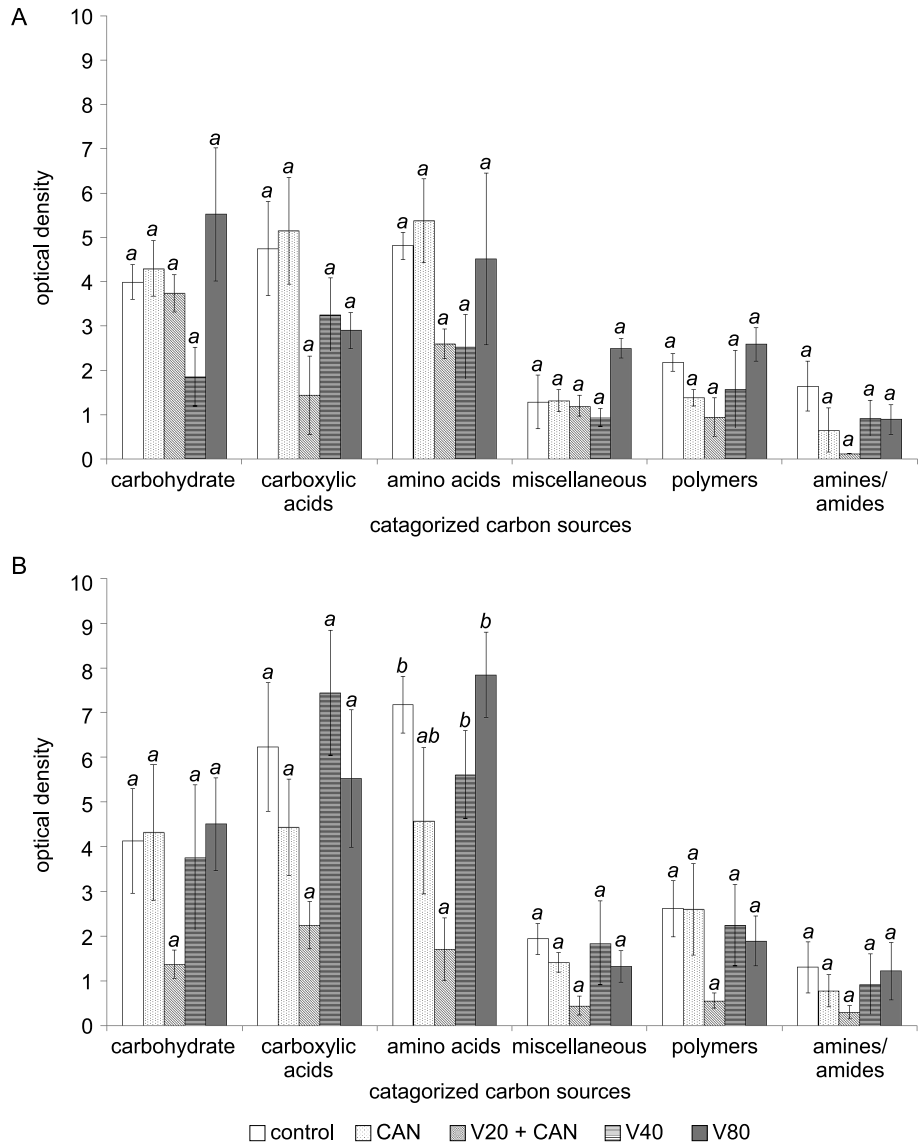

Fig. 2. Categorized substrate utilization pattern by microbial communities using BIOLOG EcoPlates™ at the start (A) and the end (B) carbohydrate and carboxylic acids – the letter above the column V20 + CAN is badly visible. Columns with different letters are significantly different at $P < 0.05$. The error bars indicate the standard error of the mean ($n = 3$); control – no fertilizer; CAN – mineral fertilizer 60 kg N ha^{-1}; V20+CAN – vermicompost 20 t ha^{-1} + + mineral fertilizer 30 kg N ha^{-1}; V40 – vermicompost 40 t ha^{-1}; V80 – vermicompost 80 t ha^{-1}

Unless an assessment dehydrogenase activity we get the results about the catabolic activity of microorganisms from the current soil available resources, the average well colour development (AWCD) reflects the oxidative capacity of soil microorganisms developing from various carbon sources in Biolog Eco-plates and may be used as an indicator of whole microbial activity (Garland, Mills 1991). At the start of the experiment the AWCD values at 96 h of incubation ranked in the following order: V20+CAN<V40<CAN< <Control<V80. The highest value of AWCD (Figure 1C) was in the vermicompost treatment V80 (OD 0.554), but the difference between V80 and the control was not statistically confirmed. We found no statistically significant differences in categorized carbon sources (carbohydrates, carboxylic acids, amino acids, miscellaneous, polymers, amines/amides) between soil treatments (Figure 2A). At the end of the experiment, the AWCD values ranked in the following order: V20+CAN<CAN<Control<V80<V40. The highest values of AWCD were in treatments V40 and V80 (OD 0.588 and 0.586, respectively), however the differences between these treatments and the control (OD 0.564) were not statistically confirmed. In soil, which was treated with vermicompost+mineral fertilizer (V20+CAN) AWCD was significantly ($P < 0.05$) lower (OD 0.144). AWCD was found to be 70%, 75.5% and 75.4% lower, respectively, in the V20+CAN compared to CAN, V40 and V80. However, of the categorised organic substrates only amino acids utilization was significantly lower ($P < 0.05$) (Figure 2B). This contrasts with Xie et al. (2009) who reported the highest AWCD in mineral fertilization (NPK) and the lowest in composted straw application soil in a long-term field experiment.

Soil nutrients, especial organic matter, are important drivers of soil microbial community composition. The Shannon index diversity and evenness indices (Table 1) of V20+CAN soil were, as well as AWCD, statistically significantly lower ($P < 0.05$) than these of other treatments during the

Table 1

Shannon diversity index, substrate evenness and richness of the microbial functional diversity

Treatments	Shannon index	Evenness	Richness	Shannon index	Evenness	Richness
	start of greenhouse experiment			end of greenhouse experiment		
Control	2.43 ± 0.04^{b}	0.80 ± 0.04^{c}	21.67 ± 3.53^{a}	2.52 ± 0.11^{b}	0.89 ± 0.01^{b}	17.00 ± 1.53^{a}
CAN	2.52 ± 0.06^{b}	0.77 ± 0.01^{bc}	27.00 ± 2.65^{a}	2.42 ± 0.09^{b}	0.80 ± 0.03^{b}	21.33 ± 3.53^{a}
V20+CAN	1.78 ± 0.03^{a}	0.61 ± 0.03^{a}	19.00 ± 1.53^{a}	1.98 ± 0.15^{a}	0.63 ± 0.03^{a}	23.67 ± 2.73^{a}
V40	2.04 ± 0.23^{a}	0.67 ± 0.06^{ab}	21.33 ± 1.76^{a}	2.56 ± 0.10^{b}	0.86 ± 0.06^{b}	20.67 ± 2.73^{a}
V80	2.41 ± 0.02^{b}	0.74 ± 0.01^{bc}	25.67 ± 0.67^{a}	2.49 ± 0.05^{b}	0.85 ± 0.02^{b}	19.00 ± 1.15^{a}

Means ± standard error are shown ($n = 3$). The different letters in a column indicate significant differences at $P < 0.05$.

Control – no fertilizer, CAN – mineral fertilizer 60 kg N ha^{-1}, V20+CAN – vermicompost 20 t ha^{-1} + mineral fertilizer 30 kg N ha^{-1}, V40 – vermicompost 40 t ha^{-1}, V80 – vermicompost 80 t ha^{-1}

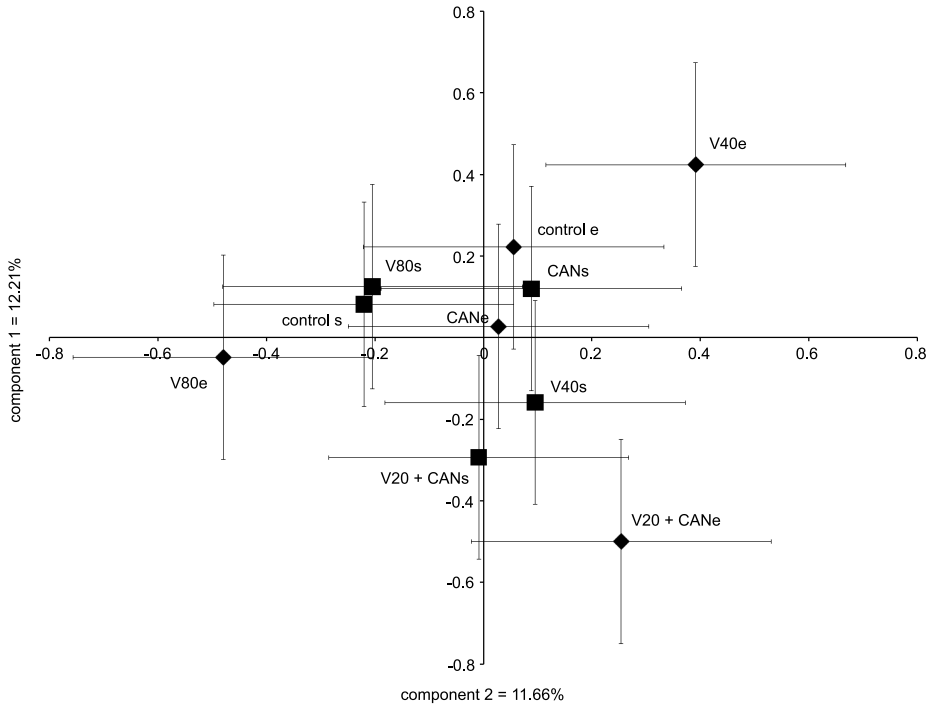

Fig. 3. Principal component analysis (PCA) of the utilization of carbon substrate from different fertilized soils. Component 1 accounted for 12.208%, and component 2 for 11.661% of the variance; control – no fertilizer; CAN – mineral fertilizer 60 kg N ha^{-1}; V20+CAN – vermicompost 20 t ha^{-1} + mineral fertilizer 30 kg N ha^{-1}; V40 – vermicompost 40 t ha^{-1}; V80 – vermicompost 80 t ha^{-1}; mark "s" – start of the experiment, mark "e" – end of the experiment

whole experiment. This indicates that the microbiota in V20+CAN had comparatively the poorest ability to oxidize the diverse C substrates tested. Campbell et al. (2003) reported that a low ability of a microbial community to oxidize diverse C substrates is indicative of its limited functional diversity. In our experiment, the number of oxidized carbon substrates (richness) was not significantly affected by different fertilization regimes (Table 1). According to the literature, vermicomposts have different chemical composition and functionality. Fernández-Gómez et al. (2011) reported that vermicomposts produced from winery wastes, olive-mill waste and damaged tomato fruits had higher microbial functional diversity than vermicompost from cattle manure. In our experiment, this could also explain the low values of the Shannon diversity index and evenness in the V20+CAN treatment.

Principal component analysis (PCA) was carried out for the observed substrate utilization patterns of all the treatments. The first and second principal components of the PCA analysis expressed 12.21% and 11.66% of the overall variance (PCA axis 1 and 2) in the five treatments. Axis 2

revealed differences in substrate utilization patterns between different fertilization treatments (Figure 3). Correlation analysis of the loadings of the most influential carbon sources on PC1 indicated that D-malic acid (0.168), D, L-α-glycerolphosphate (0.174), α-ketobutyric acid (0.182) and tween 80 (0.189) were positively correlated with PC1. The C sources with highest loadings on PC2 were D-malic acid (0.363), D, L-α-glycerolphosphate (0.339), Tween 80 (0.340) and β-methyl-D-glucoside (0.316).

CONCLUSIONS

1. Microbial biomass carbon was statistically significantly higher in variants treated with vermicompost (V40 -14.8%, V80 - 32.4%) than in the control.

2. Dehydrogenase activity was not affected by vermicompost, but we confirmed a effect of mineral fertilizer applied without vermicompost on this parameter.

3. Mixing mineral fertilizer with vermicompost had comparatively the weakest potential to oxidize diverse C substrates and affect the Shannon index diversity, but this conclusion requires further studies, specifically addressing the diversity of microbial communities by molecular methods.

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